Communications to the Editor

[Chem. Pharm. Bull. 30(7)2644—2646(1982)]

ALKYLATION OF DNA WITH A MITOMYCIN DERIVATIVE, 7-N-(p-HYDROXYPHENYL)-MITOMYCIN C. REDUCTIVE ALKYLATION AND PREFERENTIAL BINDING TO ADENINE

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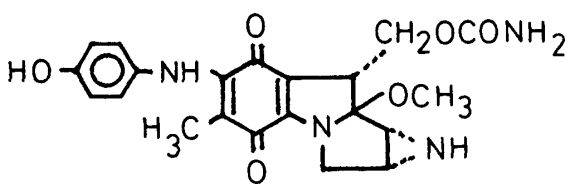
A mitomycin derivative, 7-N-(p-hydroxyphenyl)-mitomycin C (M-83) bound to DNA *in vivo* and chemically after reductive activation. Enzymatic hydrolysis of the modified DNA obtained chemically gave two modified nucleotides; 7-N-(p-hydroxyphenyl)-mitosene-deoxyadenylic acid adduct (1) and 7-N-(p-hydroxyphenyl)-mitosene-deoxyguanylic acid adduct (2). One of these modified nucleotides, 1, was identified with the modified nucleotide obtained in vivo. The preferential binding to the adenine moiety in DNA and some of chemical information on the structure of these modified nucleotides are described.

KEYWORDS-mitomycin; 7-N-(p-hydroxyphenyl)-mitomycin C; DNA; reductive alkylation; modified nucleotide

Many mitomycin drivatives were prepared to obtain more potent and less toxic antitumor agents.[1] One of attractive new antitumor mitomycin derivatives is 7-N-(p-hydroxyphenyl)-mitomycin C (m-83) which has a higher antitumor activity and a weaker toxicity than mitomycin C (MMC).[2] Alkylation of DNA with mitomycins after reductive activation is believed to be essential for the antitumor activity. So, we investigated the alkylation of DNA with M-83.

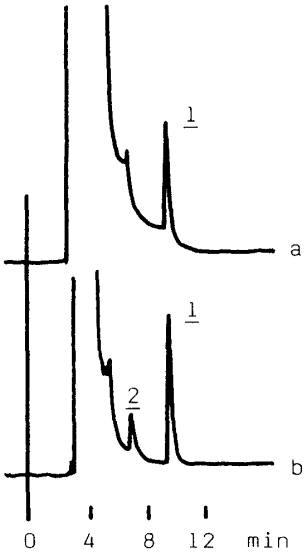
M-83 (10mg) in DMSO (0.5ml) was injected into a rat (200g) intraperitoneally. The liver was removed after 6h and DNA was extracted from the liver by Kirby's method.[3] The modified DNA was hydrolysed by nuclease P 1 and analysed by high performance liquid chromatography (HPLC, Figure 1), showing that there was a modified nucleotide, 1. The UV spectrum of 1 had maxima at 255, 337 and 387 nm. The amount of M-83 bound to DNA was about one molecule per 2-3 x 10^4 nucleotides in DNA, estimated from the peak height of 1.[4]



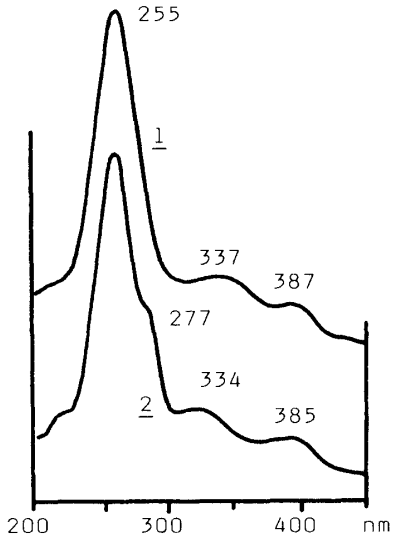
M-83

We studied the reaction of M-83 with calf thymus DNA in order to get information on the structure of the modified nucleotide, 1. Incubation of

Figure 1. HPLC and UV Spectra of Modified Nucleotides



HPLC of hydrolysate of the modified DNA a) in vivo, and b) chemically with M-83.



UV spectra of modified nucleotides, 1 and 2.

DNA with M-83 in a buffer (pH 7.5) did not cause any binding of detectable M-83 by UV spectroscopy. Reductive activation of M-83 seems to be required for the covalent binding to DNA. Recently, we reported that catalytic hydrogenation of MMC is an appropriate model reaction of *in vivo* activation of MMC.[5] Thus, M-83 (0.5mg/ml) was reduced catalytically using 5% Pd-C in the presence of calf thymus DNA (1mg/ml) in MeOH-H_2O (1:1 v/v) with H_2 gas. The reduction was stopped when the green color of the mixture turned to blue (ca. 20min at r.t.). The solution was filtered and the modified DNA was freed from noncovalently bound drugs by reprecipitation from EtOH-H_2O. The amount of M-83 bound to the DNA was about one molecule per $1\text{-}2\times10^3$ nucleotides in DNA, which was comparable to that of MMC under similar conditions, estimated from the UV spectrum of the modified DNA. The modified DNA thus obtained was hydrolysed with nuclease P 1 and analysed by HPLC (Figure 1). Two modified nucleotides, 1 and 2, were recognised. One of these was identified as the modified nucleotide 1, obtained *in vivo* by comparing their retention times on HPLC and their UV spectra. These nucleotides were different from those modified with MMC.[5] The UV spectra of 1 and 2 (Figure 1) suggest that they have a mitosene skeleton.[5] These nucleotides were purified by HPLC and were hydrolysed with 1N HCl at 100°C for 1h: Products from 1 were adenine (36%) and p-aminophenol (15%), and products from 2 were guanine (25%) and p-aminophenol (10%). All these compounds were identified by comparison of their retention times on HPLC and their UV spectra with those of authentic samples. These results suggest that the modified nucleotide 1 is a 7-N-(p-hydroxyphenyl)-mitosene-deoxyadenosine-5'-phosphate adduct with the binding site of a heteroatom of the adenine moiety, and 2

is a 7-N-(p-hydroxyphenyl)-mitosene-deoxyguanosine-5'-phosphate adduct with the binding site of a heteroatom of the guanine moiety. Considering the stability of these modified nucleotides in acidic and basic media and comparing the results we obtained concerning the structure of MMC bound nucleotides, possible binding sites are position C^1 of M-83 and position N^6 of adenine for 1 or position N^2 of guanine for 2. The results showed that M-83 binds rather selectively to the adenine moiety in DNA, while MMC binds to guanine more than to adenine. The different selectivity to bases may be attributed partially to the difference of the molecular shapes between M-83 and MMC, because the covalent binding of these drugs to DNA should be preceded by noncovalent binding, probably intercalation. The difference in therapeutic effects between M-83 and MMC also may be related to the binding selectivity.

In summary, M-83 bound to DNA *in vivo*. Reductive activation is very plausibly required for the binding which was supported by the chemical modification. The modified nucleotides were different from the MMC bound nucleotides. A more selective binding to adenine was shown.

ACKNOWLEDGMENT We are grateful to Kyowa Hakko Kogyo Co. for a generous supply of M-83.

REFERENCES AND NOTES

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(Received June 3,1982)